

were observed through cover slips at 37 C with oil immersion, bright medium contrast phase optics. A small, nonseptate cell was located and photographed on a Kodak M plate immediately after preparation of a slide and again shortly before cell division took place. The photographic plates were then positioned so that all debris was superimposed. Prints were made from these superimposed plates.

Figure 1 illustrates that *E. coli* K-12 AB1157 elongates at both ends before dividing. Figure 2 illustrates that *E. coli* K-12 AB1899, a radiation-sensitive mutant derived from AB1157, likewise elongates at both ends before division. Ionizing radiation induces the formation of long nonseptate filaments in AB1899 (Adler and Hardigree, *J. Bacteriol.* **87**:720, 1964). Figure 3 illustrates that these filaments, like the normal cells, elon-

gate at both ends. Figure 4 shows the growth pattern of *E. coli* B (ORNL). In this strain, septation occurs irregularly but elongation is always at both ends. Figure 5 demonstrates that elongation of a cell of *E. coli* B/r (ORNL), a radiation-resistant mutant of *E. coli* B, is at one end only. Several cells of each of the four strains have been observed by this technique. In *E. coli* B/r (ORNL), because of its distinct behavior, 15 cells have been photographed and show no exception to the pattern observed in Fig. 5.

The pattern of cell elongation observed for *E. coli* AB1157, AB1899, and B (ORNL) is compatible with diffuse insertion of new wall or cell-wall synthesis at both ends. The pattern of elongation observed for *E. coli* B/r (ORNL) suggests that wall synthesis and cell elongation occur at one end in this strain.

GROWTH RATES OF *STREPTOCOCCUS PYOGENES* AND DERIVED L FORM AT VARIOUS TEMPERATURES

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It has been demonstrated that conversion of a group A, type 12 streptococcus to a stable L form is accompanied by alterations in chemical composition (Panos, Barkulis, and Hayashi, *J. Bacteriol.* **78**:863, 1959), carbohydrate metabolism (Panos, *J. Bacteriol.* **84**:921, 1962), and in the accumulation of the implicated cell-wall precursor, uridine diphosphate-muramic acid-peptide (Edwards and Panos, *J. Bacteriol.* **84**:1202, 1962). However, very little is known concerning the effect of temperature upon growth (i.e., increase in numbers) of an L form. This note deals with a comparison of the growth rates at various temperatures of a group A, beta-hemolytic streptococcus and a derived stable L form obtained with the aid of penicillin.

The streptococcus and L form are the same as those employed earlier (Panos, *J. Bacteriol.* **84**:921, 1962). The medium for each of these organisms was described (Panos et al., *J. Bacteriol.* **78**:863, 1959). For turbidity determinations, 100 ml of media in 250-ml Erlenmeyer flasks equipped with side arm tubes were inoculated with 5 and 10 ml of overnight cultures of the coccus and L

form, respectively. All incubations (± 0.25 C) were performed in a thermostatically controlled water bath. Growth was determined at 20-min

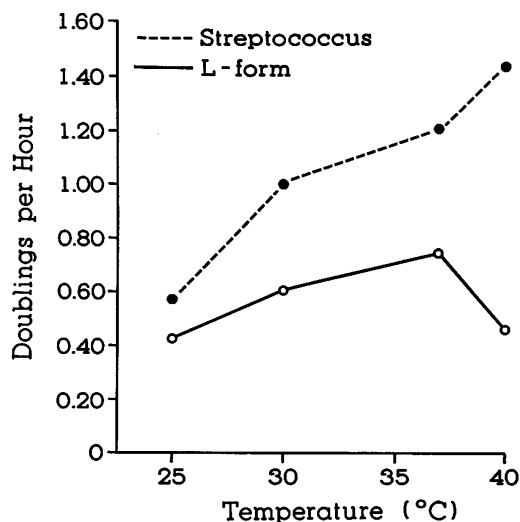


FIG. 1. Growth rates versus temperature relationship.

intervals with a Coleman model 14 spectrophotometer at 650 $m\mu$. Uninoculated media served as blanks. Doublings per hour were calculated from turbidity and viable count (Panos et al., J. Bacteriol. **80**:336, 1960) data. Each point is an average of at least two duplications.

Figure 1 illustrates the comparative relationship of doublings per hour with temperature for the parent coccus and L form. No growth of either organism occurred at 45 C. It will be noted that an increase in temperature results in an increase in the growth rate for both organisms. However, the rates for the L form remain considerably lower than those of the coccus. A marked decrease

in the growth rate of the L form at 40 C, as compared with the maximal rate attained by the coccus, points to a new variable which may be temperature-mediated. What this new variable may be indicative of is, at present, obscure. These observations, coupled with the extreme diversity of individual cell sizes consistently observed during growth of an L form, may prove useful in studies of cellular division.

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METHOD FOR ELECTROLYSIS OF CULTURE MEDIUM TO INCREASE GROWTH OF THE SULFUR-OXIDIZING IRON BACTERIUM *FERROBACILLUS SULFOOXIDANS*

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Autotrophic growth of *Ferrobacillus sulfooxidans* (Kinsel, J. Bacteriol. **80**:628, 1960) on iron as the energy source requires the oxidation of extremely large amounts of iron. Our experiments indicate a cell yield of only 1 to 2 g (wet weight) from the oxidation of 500 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. This is the harvest obtained from 20 liters of medium at a ferrous iron concentration of 5,000 ppm.

The following describes a procedure for the continual regeneration of reduced iron in culture medium which has been inoculated with iron-oxidizing autotrophs. This procedure enables one to obtain high yields of physiologically active cells with small volumes of medium.

The culture apparatus (Fig. 1) consists of a 500-ml wide-mouth Erlenmeyer flask containing 400 ml of inoculated culture medium, an aerator, and two platinum electrodes (A. H. Thomas Co., Philadelphia, Pa.; cathode electrode-8308, anode electrode-8306); one of the electrodes, the anode, is contained in a porous clay cup (Fisher Scientific Co., Pittsburgh, Pa.; 2-535-1). The clay cup is attached to the aerator with rubber bands; the other electrode, the cathode, surrounds the entire

assembly. The assembly is held in a rubber stopper cut to permit exit of an air-outlet tube, a culture (or water)-addition tube, the aerator, and the two electrodes. The apparatus is sterilized prior to the introduction of the culture. Direct

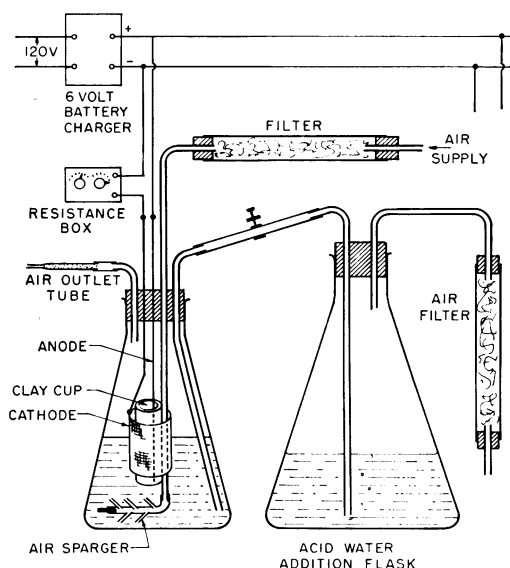


FIG. 1. Apparatus for electrolysis of iron medium.

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